J. HYM. RES.
Vol. 16(1), 2007, pp. 7–10

The Effect of Gland Secretions on Escape Chewing in *Melittobia* (Hymenoptera: Eulophidae), Including Cross-species Investigations

Leif D. Deyrup and Robert W. Matthews*

Department of Entomology University of Georgia, 413 Biological Sciences Building, Athens, GA 30602-2603 USA; email: lsdeyrup@hotmail.com; rmatthew@uga.edu
*Address for correspondence: Robert W. Matthews, Department of Entomology, University of Georgia, 413 Biological Sciences Building, Athens, GA 30602-2603; tel (706) 542-2311; fax (706) 542-2640; rmatthew@uga.edu

Abstract.—*Melittobia* is a genus of small, gregarious idiobiont parasitoids in the family Eulophidae. Following emergence as adults, females form circles in which they cooperate to chew an escape hole from the host cells in which they developed. Dry milked crude venom, which could contain constituents from the alkaline gland as well as the venom reservoir, has been shown to elicit chewing in *M. digitata*. Here we investigated whether a related species (*M. femorata*) chewed in response to compounds in its dissected venom reservoir plus alkaline-gland, and whether crude venom milked from a member of another species group (*M. australica*) would also elicit chewing in *M. digitata*. *Melittobia femorata* chewed significantly more at combined gland and reservoir extract-marked spots than at controls. To examine the crude venom's effect across species we marked spots with milked *M. australica* venom, and introduced female *M. digitata* wasps. These milked crude venom spots elicited chewing similar to that elicited by that of *M. digitata* marked spots, and the response to either's venom was significantly different from blank controls. Possible reasons for the lack of a high level of specificity in the chewing response to a pheromone are discussed.

Melittobia Westwood is a cosmopolitan genus of small gregarious parasitic wasps (Balfour-Browne 1922, Buckell 1928, Dahms 1984b). They are commonly found attacking mud dauber (Hymenoptera: Sphecidae) prepupae and their associates (Matthews 1997), but also attack a wide range of solitary bees and wasps and their associates (Balfour-Browne 1922, Krombein 1967).

When attacking a mud dauber wasp they have to escape from the thick-walled mud nest, yet females do not have noticeably well developed mandibles. Donovan (1976) observed *M. hawaiiensis* Perkins females circled around another female that had started chewing a pit in the mud wall, and speculated that they then cooperated in chewing their way out. Subsequently, such cooperative chewing has been observed in several *Melittobia* species (L.D. Deyrup unpublished).

Deyrup et al. (2005) reported that chewed pits invariably had associated sting marks and showed that a putative pheromone in the milked crude venom, which most likely contains constituents of the alkaline gland as well as the venom reservoir, of *M. digitata* Dahms elicited chewing from conspecific females. Because similar chew pits made by other species of *Melittobia* also typically show sting marks in their centers (Deyrup unpublished), we decided to investigate whether extracted venom components would elicit chewing in a closely related species, *M. femorata* Dahms (Dahms 1984a).

Such chewing, if demonstrated, could be in response to a normal constituent of crude venom, or blend of odors. Regardless, it is difficult to envision selection pressure sufficient to cause evolutionary divergence in such a cue, since there appear to be no negative effects of co-

operative escape chewing, even among unrelated females.

METHODS

In general the methods follow those described by Deyrup et al. (2005). *Melittobia australica* Girault responded to the venom-milking procedures described in Deyrup and Matthews (2003), yielding adequate amounts of crude venom for the experiment. However, *M. femorata* does not respond to this venom-milking technique. Therefore, as an alternative we dissected the lower reproductive tract of females in insect saline [10 mM sodium phosphate, 0.9% (w/v) NaCl, pH 8.0]. While there are many possible pheromone sources in the female reproductive system, the two most likely are the alkaline gland and venom reservoir. These were separated from the ovipositor and combined for use in the experiment. Since milked crude venom used in previous work could contain a combination of the fluids contained in both organs we decided to combine them for this experiment.

As described in Deyrup et al. (2005), 20 plastic box lids were prepared for the first set of experimental treatments by making four pin indentations, one in each corner of the inner side. We then smeared the combined alkaline gland and venom reservoir dissected from a single female of *M. femorata* into one pin indentation and repeated this using a fresh female applied to the pit on the opposite corner. The other two pits served as controls for chewing stimulated by the pit alone as in Deyrup et al. (2005). Treated lids were then placed on 20 boxes of 250–300 1–3 day old mated *M. femorata* females and left for 12 hours in complete darkness at 25 C, after which they were examined for evidence of chewing at each of the four pits.

To determine if *M. australica* or *M. digitata* would be stimulated to chew by *M. australica* crude venom, we set up a two more series of boxes. Three corner circles were drawn on the lids as in Deyrup et al. (2005) and randomly assigned one of three treatments. One circle received 1 FED (female equivalent dose) of milked *M. australica* venom. In another circle a clean pin rub served as a negative control, and the third circle was 1 FED of milked venom from a *M. digitata*. Fifteen of these lids were prepared for each series, and placed on boxes of 250–300 females as before. Boxes were then placed in absolute darkness at 25 C, and scored for signs of chewing 12 hours later.

Cochran Q tests were used to analyze chewing frequencies (Statistica 6.0). This test was chosen because the treatments were paired, and the results were scored as chewing presence or absence (1 or 0 respectively).

RESULTS

The experimental group containing smeared *M. femorata* venom reservoir and alkaline gland contents elicited chewing from *M. femorata* in at least one of the two treated pits in 19 of the 20 replicates. In contrast, both control pits were chewed on only two occasions out of 20. These differences were highly significant ($P < 0.0001$, $Q = 17.0000$, 1 df). In the series to determine if *M. australica* chewed at their own milked crude venom or the milked crude venom from *M. digitata*, there was no chewing what-so-ever at any treatment or control.

In the experiment to examine if chewing was elicited in *M. digitata* by milked crude venom from *M. australica*, chewing occurred in 9 of the 15 replicates (Table 1). The overall Cochran test was significant ($P < 0.0031$, $Q = 11.5556$, 2 df). Therefore, using Fisher's test for multiple analyses, we ran pairwise Cochran tests that revealed a significant difference between the blank and *M. australica* venom ($P < 0.0047$, $Q = 8.0$, 1 df), and the blank and *M. digitata* venom ($P < 0.0143$, $Q = 6.0$, 1 df). There was no significant difference between chewing at the positive control, *M. digitata* venom,

Table 1. Chewing response by *M. digitata* females after 12 hours exposure of treatment circles containing venom milked from either *M. digitata* or *M. australica*. Different letters in the "Significance" column indicate significant differences using a Cochran Q test $p < 0.05$, df = 1 (Statistica 6.0).

Treatment	Chewed	Replicates	Significance
M. australica venom	9	15	a
M. digitata venom	7	15	a
Control	1	15	b

and *M. australica* venom ($P < 0.3173$, $Q = 1.0$, 1 df).

DISCUSSION

The *M. femorata* chewing results in response to dissected *M. femorata* reproductive tract organs suggest that *M. femorata* has a pheromone in its crude venom that stimulates chewing at a particular spot. This adds support to the idea that chewing in response to crude venom components evolved before the speciation event that separated *M. digitata* and *M. femorata*.

The negative results for *M. australica* chewing are hard to interpret since the design does not allow us to test a "lack of stimulus". The species has been observed to cooperatively chew. There could be many reasons for the crude venom not to be attractive such as the possibility that other factors are necessary or that chewing only occurs during a particular unestablished window of opportunity. More rigorous experimentation would be required to establish that the crude venom is not at least a part of the chewing stimulus.

The positive results for the attraction of *M. australica* crude venom for *M. digitata* females (Table 1) might seem surprising since the two species belong to different species groups (Dahms 1984a). Especially since we were unable to elicit chewing in response to milked crude venom for *M. australica*. However, there is little reason to expect that such a pheromone, if there is a pheromone for chewing in *M. australica*, would not be conserved, since a mutation could leave carriers trapped in the host's cell. Even if there is no such pheromone present in crude venom for chewing in *M. australica*, the chemical that stimulates chewing for *M. digitata* could be one that is stable and under selection for another purpose (e.g., perhaps containing a constituent causing developmental delay in the host [Deyrup et al. 2003]). Components of other pheromones appear to have been conserved in *Melittobia*. Matthews et al. (1985) found that females of *M. digitata*, *M. femorata*, and *M. australica* were attracted to non-conspecific as well as conspecific males in choice tests. Further work should be done on investigating the source of the pheromone in which either the venom reservoir or the alkaline gland is presented alone and together.

ACKNOWLEDGEMENTS

Mark Deyrup, Jan Matthews, and Jorge González provided valuable discussion and editorial help. This work was supported in part by a grant from the National Science Foundation to R. W. Matthews.

LITERATURE CITED

Balfour-Browne, M. A. 1922. On the life-history of *Melittobia acasta* Walker, a chalcid parasite of bees and wasps. *Parasitology* 14: 349–369.

Buckell, E. R. 1928. Notes on the life-history and habits of *Melittobia chalybii* Ashmead (Chalcidoidea: Elachertidae). *Pan-Pacific Entomologist* 5: 14–22.

Dahms, E. C. 1984a. Revision of the genus *Melittobia* (Chalcidoidea: Eulophidae) with the description of seven new species. *Memoirs of the Queensland Museum* 21: 271–336.

———. 1984b. A review of the biology of species in the genus *Melittobia* (Hymenoptera: Eulophidae) with interpretations and additions using observations on *Melittobia australica*. *Memoirs of the Queensland Museum* 21: 337–360.

Deyrup, L. D. and R. W. Matthews. 2003. A simple technique for milking the venom of a small parasitic wasp, *Melittobia digitata* (Hymenoptera: Eulophidae). *Toxicon* 42: 217–218.

———, M. Deyrup, and R. W. Matthews. 2003. Paralyzation and developmental delay of a factitious host by *Melittobia digitata* (Hymenopter: Eulophidae) *Journal of Entomological Science* 38: 703–705.

———, R. W. Matthews, and J. M. González. 2005. Cooperative chewing in a gregariously developing parasitoid wasp, *Melittobia digitata* Dahms, is stimulated by structural cues and a pheromone in crude venom extract. *Journal of Insect Behavior* 18: 293–304.

Donovan, B. J. 1976. Co-operative material penetration by *Melittobia hawaiiensis* (Hymenoptera: Eulophidae) and its adaptive significance. *New Zealand Entomologist* 6: 192–193.

Krombein, K. V. 1967. *Trap-nesting Wasps and Bees: Life Histories, Nests, and Associates*. Smithsonian Press, Washington, DC.

Matthews, R. W. 1997. Teaching ecological interactions with mud dauber nests. *The American Biology Teacher* 59: 152–158.

———, J. Yukawa, and J. M. González. 1985. Sex pheromones in *Melittobia* parasitic wasps: Female response to conspecific and congeneric males of 3 species. *Journal of Ethology* 3: 59–62.